AN ELECTRON MICROSCOPIC STUDY OF BLISTER FORMATION IN ERYTHEMA MULTIFORME*

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The etiology of erythema multiforme (1, 2) has been the subject of speculation for many years. Bacteria, virus and immunologic mechanisms have all been put forward (3, 4). The latter is probably more generally accepted as the cause at the present time. A number of drugs have been implicated as have other agents (3, 4).

The histology of erythema multiforme is complicated by the wide variation in severity and the course of the disease. Since the lesions clinically go through a cycle of formation, spread, central healing and not infrequently reappearance in the central healing zone, marked differences in the histologic appearance in any given lesion are to be expected (1).

The dermal changes seen with the light microscope include dermal edema, perivascular lymphocytic infiltration and occasionally eosinophils and neutrophils. In severe cases endothelial cell changes extending to necrosis may be seen. Fibrinoid necrosis involving vessels and perivascular connective tissue has been reported (5). The bullae are of a subepidermal variety. In the early stages of blister formation there is minimal epidermal cell alteration while in later stages the epidermal roof of the blister usually shows signs of marked damage (1).

We have attempted to describe the lesions of bullous erythema multiforme, both acute and chronic, as seen with an electron microscope.

METHODS AND MATERIALS

Five patients with bullous erythema multiforme were investigated in this study. Two of the patients were middle-aged people with a definite history of drug consumption prior to the onset of bullae. One was a child, three years of age, with a severe lethal bullous reaction to chloromycetin. Biopsy specimens were obtained immediately before the administration of corticosteroids and after five days of intensive therapy. In two cases, no history of a particular etiologic agent could be elicited. One of these was a thirty-two year old man with mild, chronic erythema multiforme while the remaining case was a severe but chronic type of bullous erythema multiforme in a sixty-five year old patient.

Only small blisters, measuring not more than 2 to 3 mm. in diameter were taken for biopsy study. As far as possible, only lesions of very recent origin were used. In the first three cases the biopsy material was removed by a manual punch under a minimum amount of novocain anesthesia. In the last two cases the blisters were removed by a motor-driven rotary punch without local anesthesia. The punch biopsy specimens were immediately fixed in osmium tetroxide, subjected to dehydration by alcohol and embedded in n-butyl methacrylate (6, 7).

RESULTS

The alterations in erythema multiforme, as the name implies, showed considerable variations. By limiting our examination to bullous lesions, one facet of the clinical morphology was constantly present. It was to be expected, however, that the different clinical courses of our patients would be reflected in variations of the histologic appearance of the lesions. Thus no two of the cases were exactly alike morphologically, but a number of changes were consistently present in variable degree. In order to have comparable lesions the age and rate of progress of the disease is important and this is not easily controlled. As a result of these factors, some of the lesions showed healing as well as progression of the disease.

Figure 1 illustrates a section of normal skin at the dermal-epidermal junction. The collagen fibers were densely arranged close to the basement membrane. Fine, discrete filaments extended from the basement membrane downwards to the uppermost layer of collagen fibers with which they connected. Adjacent to the junction granules at the base of the basal cells there was a modificaton of the basement membrane. In a few areas (Fig. 1), basement membrane material was in intimate contact with the cell membrane, whereas there was usually a a definite space of narrow width between the

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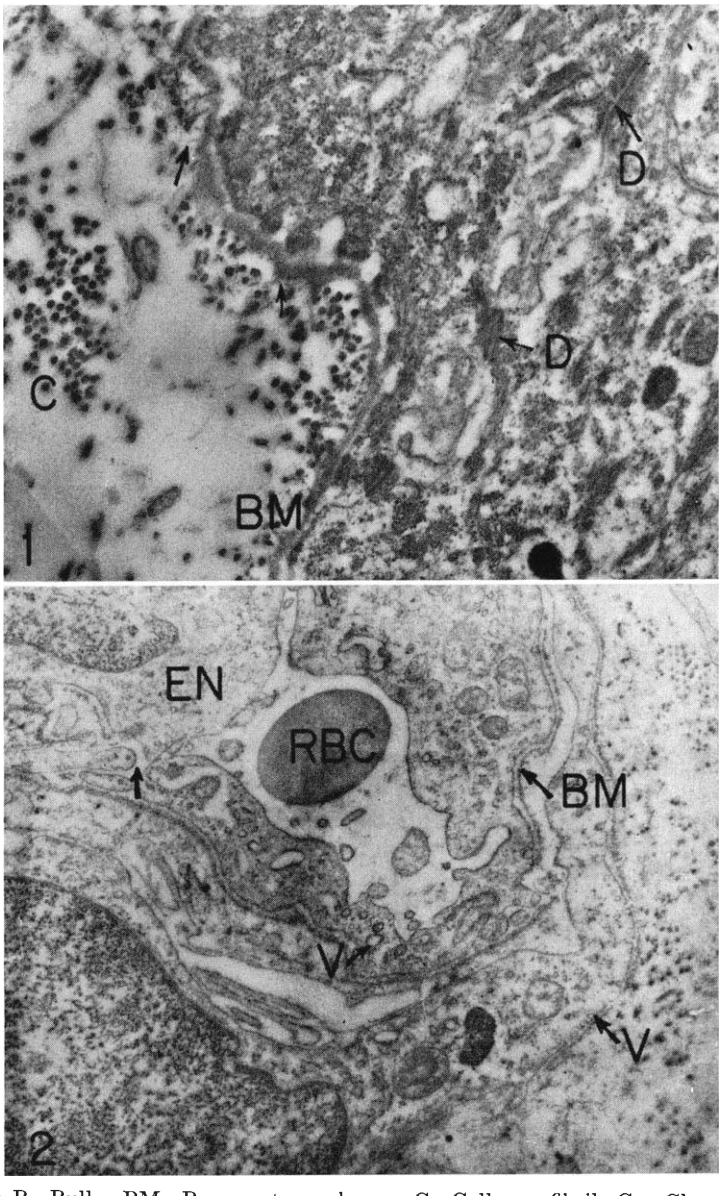

Abbreviations: B—Bulla; BM—Basement membrane; C—Collagen fibril; Cr—Chromatin; CP—Cell process; D—Desmosomes; DC—Dermal cell; De—Dermis; E—Elastic tissue; EC—Epidermal cell; F—Fibrin; IS—Intracellular space; L—Lipid body; M—Mitochondrion; MG—Melanin granule; N—Nucleus; NF—Nerve fiber; P—Proteinaceous debris; PP—Plasma protein; RBC—Red blood cell; T—Tonofilament; V—Vesicle.

FIG. 1. This is a section of normal skin at the dermal-epidermal junction. Numerous collagen fibrils (C) provide the loose structure of the dermis. There is a concentration of collagen fibers immediately below the basement membrane (BM) which is intact. Fine discrete filaments (arrows) extend from the basement membrane downward and connect with the uppermost located collagen fibers. The slight variations in thickness of the basement membrane are due to tangential sections of the curving membrane. The basement membrane is generally separated from the plasma membrane by a narrow space of relatively constant width. However, in the regions of the basally located junction granules the basement membrane frequently is in contact with those parts of the plasma membrane which participate in the formation of the junction granule. Lateral desmosomes (D) of the basal cells are present. × 11,400

FIG. 2. This small venule in the superficial dermis demonstrates separation of the endothelial cells (unlabelled arrow). This separation associated with the clear spaces between the two layers of the basement membrane (BM) is usually associated with outward passage of fluid. Only a small portion of the erythrocyte (RBC) is included in the picture accounting for the small size. Large numbers of small vesicles (V) are present within the vascular cells. × 7,600

cell membrane and the basement membrane. The area of contact did not involve the entire area of the junction granule.

In areas where blisters had begun to form, the small veins of the dermis frequently showed the changes associated with outward fluid passage (Fig. 2) (8). There was separation of endothelial cells. Clear spaces between the two layers of the vascular basement membrane could be observed. Large numbers of small vesicles were visible within the vascular cells. The degree of alteration from vessel to vessel and case to case was variable. Associated with the vascular changes were different degrees of dermal edema reflected in a distortion of the normal spatial relationship of the collagen fibers (Fig. 3). In three of the cases osmiophilic amorphous deposits of proteinaceous material were present. These deposits were usually on and between collagen fibrils and occasionally seemed to be on the surface of cells (Fig. 3). In one fortuitous case the dense material was sufficiently well oriented to see 220 Å banding which is quite typical of fibrin (9, 10). On the strength of this banding it is safe to conclude that these deposits between and on collagen fibrils contain some fibrin (Fig. 4).

There were two predominant varieties of infiltrating cells present. Lymphocytes were seen routinely in all cases. These cells were no different than those described in the circulating blood (11). The second cell, as yet unidentified, was rather large (Fig. 5). The cytoplasm contained a great deal of rough surface endoplasmic reticulum. The arrangement of this reticulum was similar to that seen in a fibroblast and quite dissimilar to that of a plasma cell. There was little smooth surface endoplasmic reticulum. The nucleus occupied a small portion of the cytoplasm and was composed of evenly disposed nucleoplasm. On several occasions these cells were seen passing through the basement membrane. In view of the lack of characteristic granules, these cells cannot be neutrophils, eosinophils or basophils. The amount of cytoplasm and its contents makes it unlikely that these cells are lymphocytes or monocytes. The size, nuclear configuration and motility would indicate that they are the cells termed histiocytes in hematoxylin and eosin preparations. The presence of rough surface endoplasmic reticulum which would confer a degree of basophilia on staining with hematoxylin is compatible with previously designated histiocytes (12). Eosinophils were present in some of the cases within the dermis and epidermis. Neutrophils were not seen.

The basement membrane varied from block to block within a given 3 mm. biopsy specimen. At the base of a bullous region there generally existed some alteration of the basement membrane and the most severe changes were always associated with bullae. The membrane was frequently thinned and occasionally absent for short distances (Fig. 6). The fine, discrete filaments connecting the basement membrane to the dermis seemed to be decreased in number. This thinning and disruption of the basement membrane was generally associated with severe dermal edema. Adjacent to bullae the basement membrane was normal in some areas but appeared thin in other regions. However, no discontinuities were noted in the presence of normal epidermis.

In areas of healing, as indicated by the presence of basal cells in mitosis or by reepithelialization of the floor of the bullae, the basement membrane frequently consisted of 3 or 4 layers (Fig. 7). This change was particularly prominent in the one case treated heavily with steroids. The biopsy specimen taken prior to steroid therapy showed a few areas of basement membrane reduplication but not to the extent seen after therapy. The layers of the basement membrane seemed to form within a mesh work of filamentous material present in large quantities in these healing areas (Fig. 8).

The epidermal cells were as variably involved as the other components of the lesions. In areas immediately adjacent to the bullae the epidermis frequently appeared normal, but sometimes showed varying degrees of both intracellular and intercellular edema. Fig. 9 demonstrates a small focus of epidermal cells that are severely damaged. Though the cells at either extreme of this focal area of degeneration are normal, nothing can be said about the presence of a bulla above or below the plane of this section. However, the lateral separation of these damaged cells was not accompanied by aggregation of the tonofilaments as is seen in other bullous conditions such as pemphigus (13). The desmosomes at the base of some of the basal cells (i.e., the junction granules) had lost some structural components and thus were not intact. They were frequently also reduced in number in a given area.

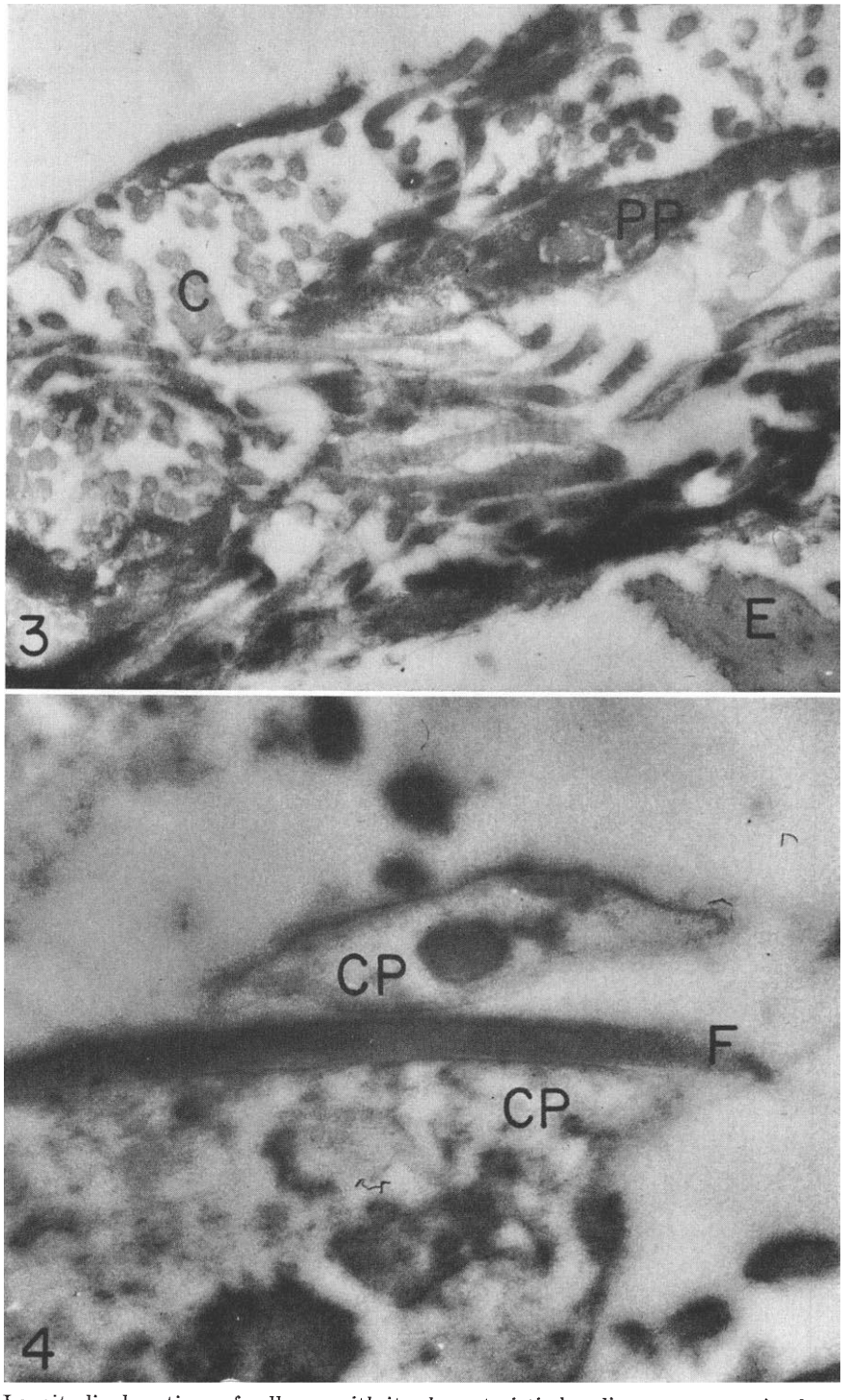

FIG. 3. Longitudinal sections of collagen with its characteristic banding are present in the center of this picture. Throughout both the longitudinal and cross sectioned collagen (C) a finely fibrillar dense material is present (PP). This represents a deposition of plasma proteins. A small segment of elastic tissue (E) can be seen in the lower right corner. × 35,880

FIG. 4. Between two cell processes (CP) a segment of deeply staining cross banded material can be seen. The periodicity is about 220 Å which is characteristic of fibrin. This particular bit of plasma protein is sufficiently well oriented to see the banding, an uncommon occurrence in tissue section. It resembles in most respects the deposits in and on the collagen seen in Fig. 3. × 41,400

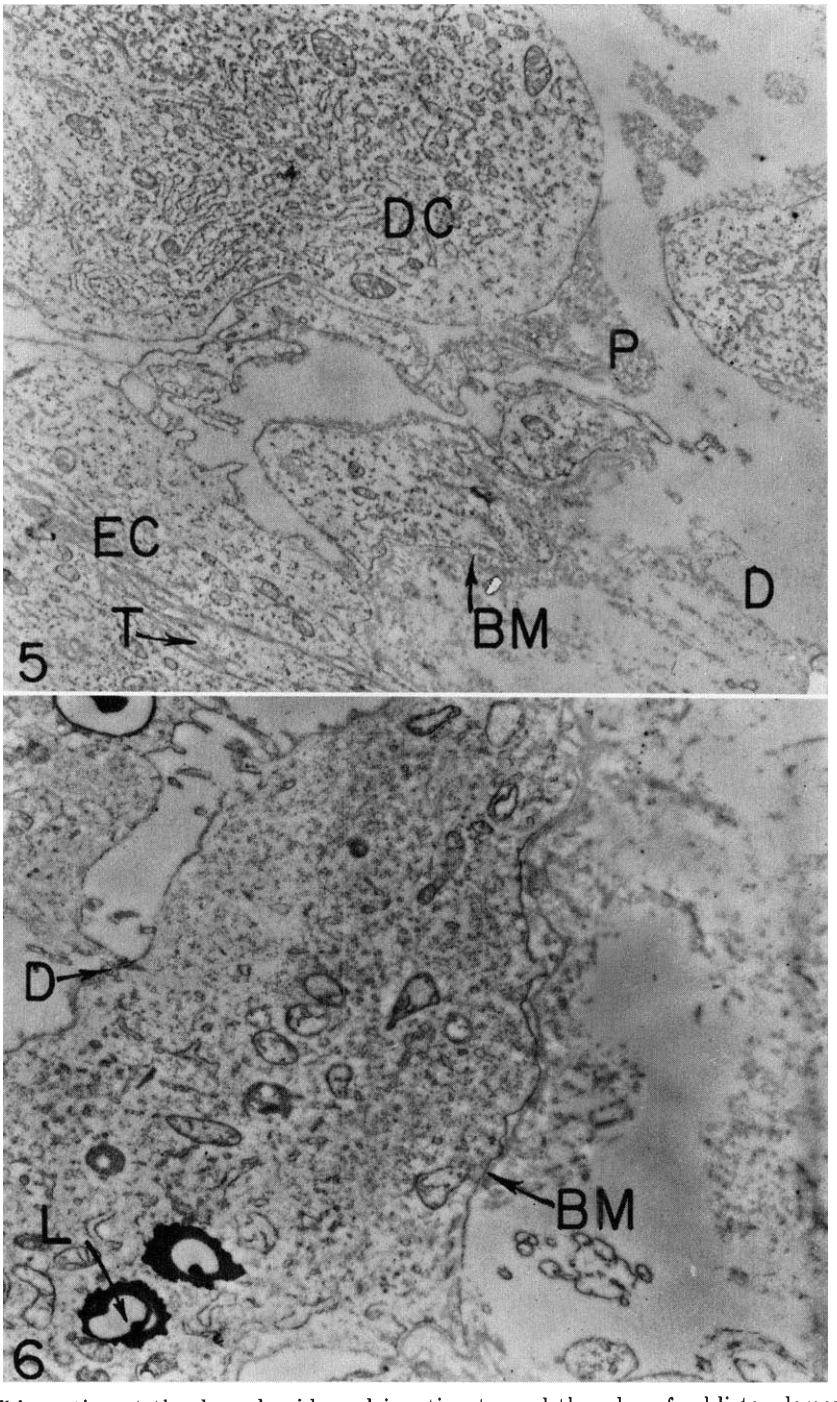

FIG. 5. This section at the dermal-epidermal junction toward the edge of a blister demonstrates a large defect in the basement membrane probably associated with the passage of the large dermal cell (DC). This ameboid cell is not identified. The tonofilaments (T) of the epidermal cells are visible, but much decreased in number. All of the desmosomes of the epidermal cells have disappeared. The dermis (D) is very edematous and proteinaceous debris (P) is visible. × 9,680

FIG. 6. Near the edge of a bulla the basement membrane (BM) at the arrow is discontinuous. The dermis is edematous. A remnant of a desmosome (D) is visible with a few adjacent tonofilaments. The majority of desmosomes are absent. The intercellular spaces are widened producing spongiosis. Lipid bodies (L) are occasionally present in normal basal cells, but their number and size are increased in this picture. The fine filaments connecting the basement membrane to the dermis appear decreased in number. × 10,560

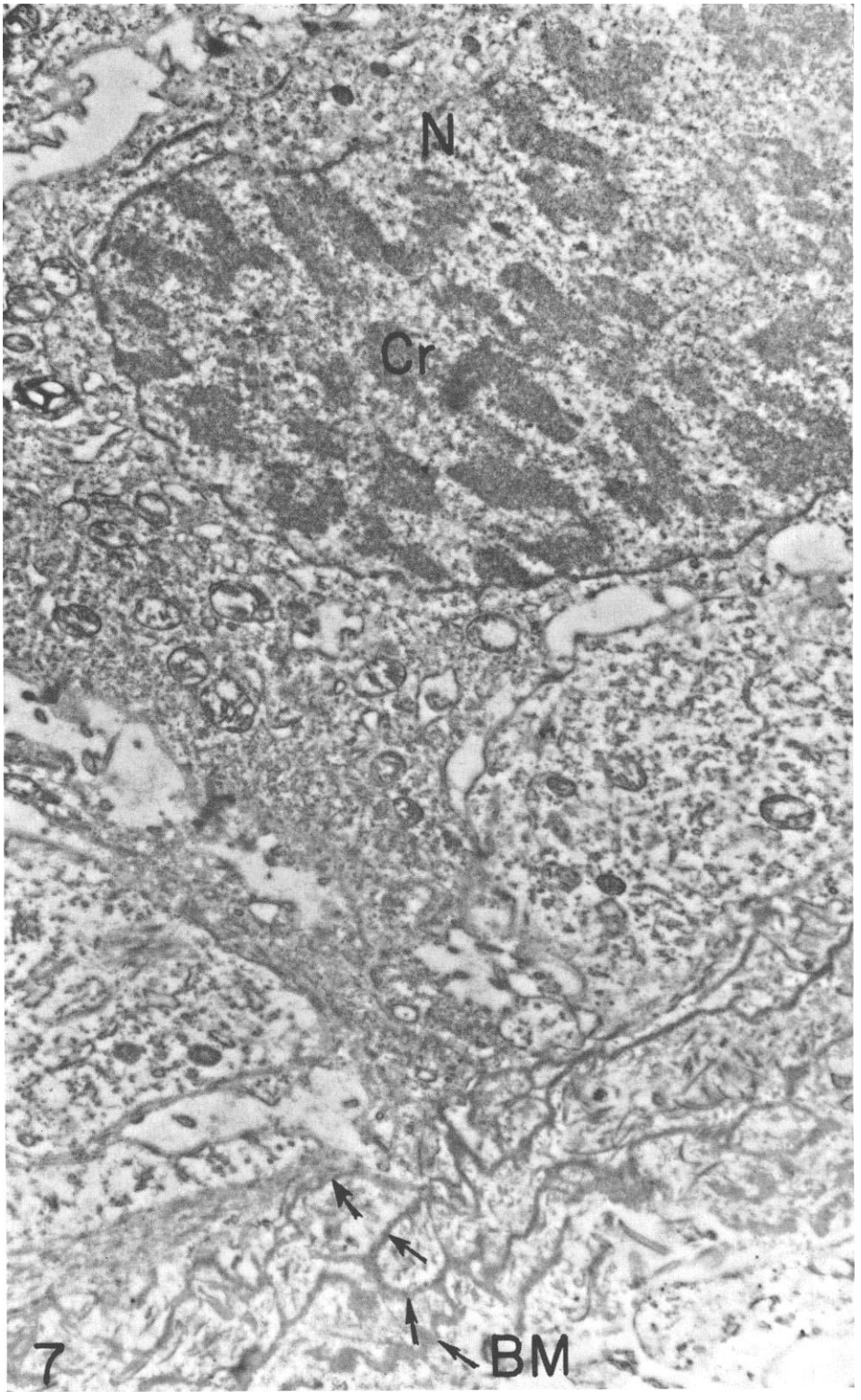

FIG. 7. This basal cell in an area of regeneration is in prophase. The nucleus (N) contains clumped chromatin (Cr). Reduplication of the basement membrane (BM) is clearly evident at the arrows. The meshwork of fine amorphous material in the region of the basement membrane is increased. × 15,700

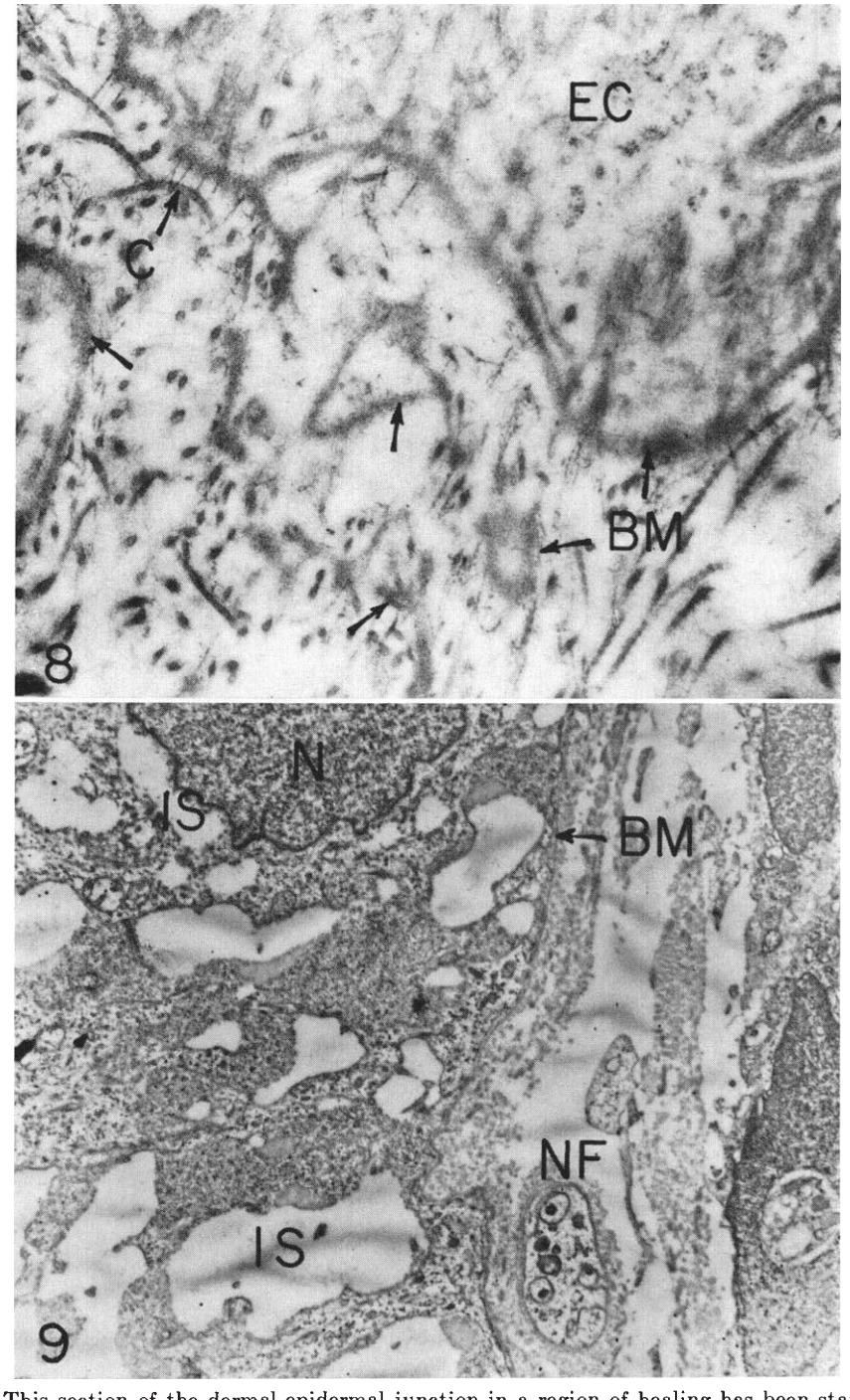

FIG. 8. This section of the dermal-epidermal junction in a region of healing has been stained with uranyl acetate to accentuate the fibrillar components. Banded collagen (C) connected to the basement membrane by fine discrete filaments is visible at C. In contrast the fibrillar meshwork of the reduplicated basement membrane is most prominent at the arrows. Very little of the basal cell (BC) is visible. Desmosomes though not present in this area of the cell, are visible elsewhere. × 30,800

FIG. 9. Large spaces (IS) within the epidermal cells as well as swollen mitochondria, loss of desmosomes and tonofilaments indicate the presence of fairly severe cellular damage prior to the onset of bulla formation. The extracellular spaces are greatly enlarged, presumably due to fluid. The dermis is edematous. A small nerve appears normal (NF). × 10,560

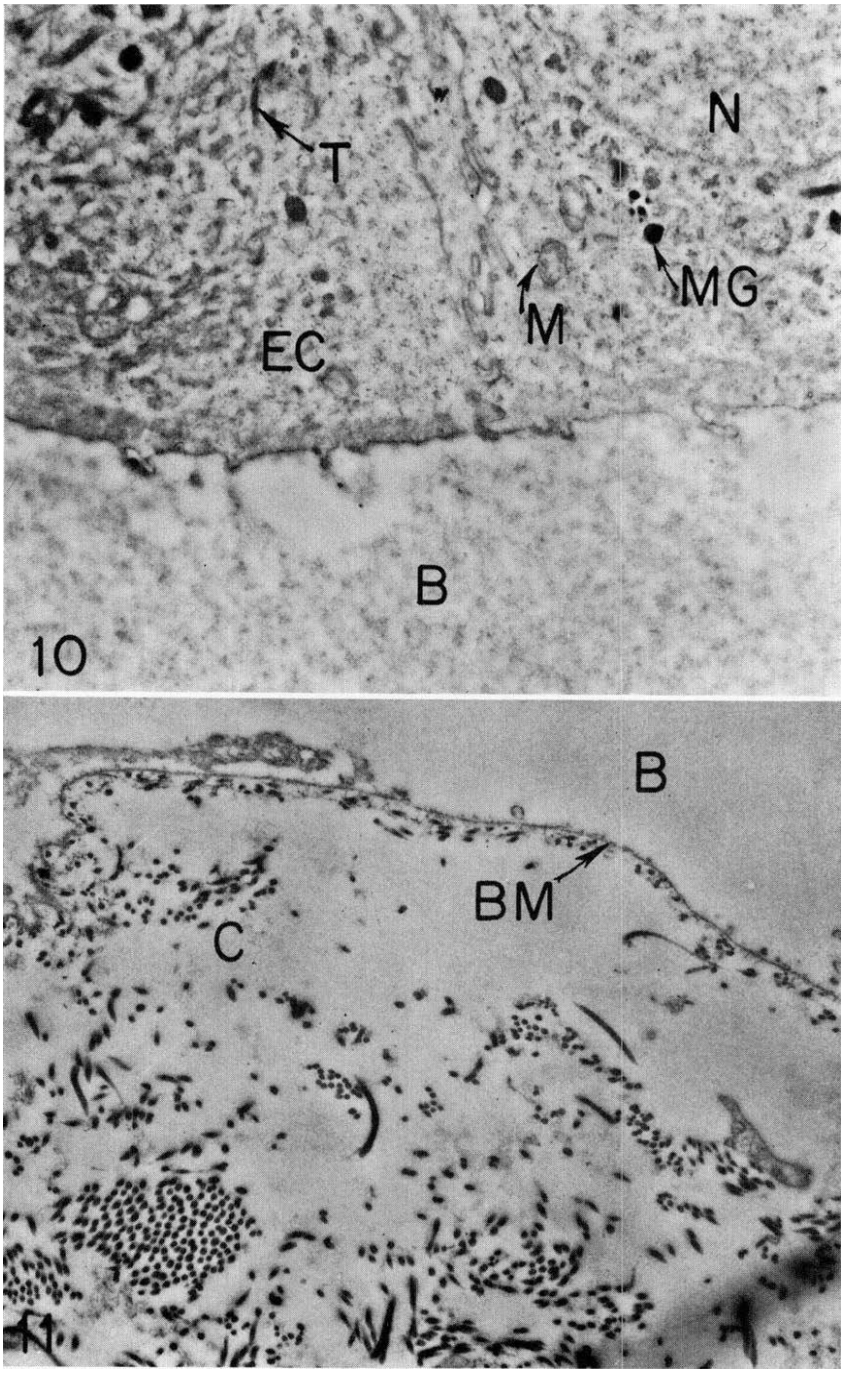

FIG. 10. This area is taken from the roof of an early bulla. The cavity of the bulla (B) contains amorphous material presumably protein in nature. The epidermal cells are not entirely normal in this part of the epidermal roof of the bulla. Melanin granules (MG), mitochondria and tonofilaments are of ordinary appearance. The nucleus (N) is within the limits of normal. × 8,800

FIG. 11. This area from the base of a blister clearly shows that the basement membrane (BM) remains with the dermis. There is a marked degree of collagen (C) separation associated with dermal edema. Above the basement membrane debris that in some cases contains recognizable cellular constituents can be seen. In this area there is no evidence of epidermal or dermal healing. × 11,880

The epidermal cells at the roof of the bullae occasionally showed remnants of junction granules. Here, too, the amount of intercellular and intracellular edema varied (Fig. 10). An occasional basal cell appeared to be partially torn from the basement membrane. In such instances recognizable cytoplasmic constituents remained on the basement membrane while the major portion of the cell adhered to that part of the epidermis that formed the roof of the bullae.

Fig. 11 demonstrates clearly that the basement membrane remains with the dermis and forms the floor of the bulla.

DISCUSSION

The primary lesion of erythema multiforme appears to involve the dermis. There are widespread vascular changes with a great deal of edema formation and collagen alteration throughout the dermis. The presence of banded fibrin associated with osmiophilic amorphous material indicates at least partial origin from intravascular protein, but other plasma components may also contribute to the formation of this material. The alteration of collagen and admixture with fibrin and plasma proteins is suggested as one mechanism for the occurrence of so-called "fibrinoid necrosis". In the subepidermal type of blister formation, the basement membrane—whether altered or not—stays with the dermis and forms the floor of the bulla. The most severe lesions of the basement membrane, including discontinuities, occur in areas of most marked edema formation at the base of the blisters. Thus it appears that the the changes in the basement membrane occur simultaneously with the dermal lesions. However, the possibility that the lesions of the basement membrane occur following the dermal alterations cannot be definitely excluded. Replacement of the epidermis at the floor of the bulla begins before the basement membrane undergoes reduplication and becomes layered in appearance. Where a multilayered basement membrane is present, there is always an epidermal covering of healthy-appearing cells. The frequent lack of a complete complement of junction granules as well as the presence of a single thin layer of basal cells seems to indicate that the epidermal cells covering the multilayered basement membrane are regenerative. The origin of the new components of the basement membrane is not clear. It is suggested that the basement membrane in the regions of multiple layering forms through condensation of the large clusters of a filamentous meshwork. These clusters of a filamentous meshwork have not been seen in normal skin or in any other of the skin diseases studied so far. Similarly, the reduplicated multilayered basement membrane has not been seen previously. It is most probable that as more skin lesions are studied a similar mode of repair of the basement membrane will be encountered.

Adjacent to the bullae, the epidermis is frequently normal. Whenever there are epidermal cell alterations in the absence of bullae, they appear to be associated with more severe dermal changes. The most severe epidermal cell changes in nonbullous areas are seen in focal areas of epidermal edema. These epidermal cell alterations include intracellular edema, loss of junction granules and dissolution of tonofilaments. The initiation of bulla formation might occur either concomitant with the dermal lesions, or it might take place as a phenomenon secondary to the dermal alterations. Since the early focal epidermal lesions are associated with advanced dermal lesions, one is led to assume that these focal epidermal changes occur subsequent to the damage in the dermis. While the exact time sequence of events is not yet clear, it was observed that in areas of severe dermal injury focal epidermal cell damage may occur prior to the onset of local blister formation. It is speculated that this focal damage in the epidermis may form the nidus for the formation of the bullae whence the enlargement of the blister might take place by lateral extension of less severely damaged epidermal cells. Lateral extension of the bullae involves two methods of separation of epidermal cells from the basement membrane. In some regions the entire epidermal cell is lifted off the basement membrane. In other areas the plasma membrane and a small amount of cytoplasm remain attached to the basement membrane in which case the remainder of the cell is torn away and lifted up in the formation of the roof of the bulla. An occasional epidermal cell is free in the blister, but the vast majority maintain lateral coherence and an entire sheet of epidermal cells form the roof of the bulla. Although we attempted to select bullae of assumed recent origin, the epidermal cells forming the roof of the blister were not entirely normal. Besides the variable intercellular and intracellular edema, an occasional severely damaged cell was present in the roof of the blisters studied.

The mode of blister formation in erythema multiforme is different from the mode of blister formation in pemphigus vulgaris. In pemphigus, the primary changes as seen with the electron microscope are in the epidermal tonofilaments, while in erythema multiforme the earliest lesions are found in the dermis. These dermal changes are more easily seen in an electron microscope than in the light microscope. The collagen changes have so far not been observed in any of the cases of pemphigus which we have studied. Thus, the absence of dermal changes in pemphigus and their presence in bullous erythema multiforme offered a distinct contrast in our series of cases.

SUMMARY

The primary lesion of erythema multiforme appears to be in the dermis. As a result of an unknown reaction dermal edema forms. Collagen fibers and individual fibrils are swollen. This is accompanied by deposition of plasma proteins on and between the collagen fibers. Basement membrane thinning and actual disruption is followed by a healing phase involving reduplication and layering of the membrane. Focal degeneration of epidermal cells and bulla formation occur in regions of severe dermal lesions. It is postulated that the formation of blisters in erythema multiforme is a consequence of the dermal alterations with subsequent loss of cohesion between the basement membrane and damaged epidermal cells.

REFERENCES

1. Lever, W. F.: *Histopathology of the skin.* Philadelphia, Lippincott Co., 2nd Ed., 1954.
2. Allen, A. C.: *The skin: A clinicopathologic treatise.* St. Louis, C. V. Mosby Co., 1954.
3. Andrews, G. C.: *Diseases of the Skin.* Philadelphia, W. B. Saunders Co., 4th Ed., 1954.
4. Rathson, M. J., Carlisle, J. W., Lee, R. E., Jr., Vernier, R. L. and Good, R. A.: Lupus erythematosus and Stevens Johnson syndrome. *A. M. A. J. Dis. Child.*, **101:** 725, 1961.
5. Alexander, M. K. and Cope, S.: Erythema multiforme exudativum major. *J. Path. Bact.*, **68:** 373–380, 1954.
6. Palade, G. D.: A study of fixation for electron microscopy. *J. Exp. Med.*, **95:** 285, 1952.
7. Caulfield, J. B.: Effects of varying the vehicle for OsO_4 in tissue fixation. *J. Biophys. Biochem. Cytol.*, **3:** 827, 1957.
8. Majno, G. and Palade, G. E.: Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: An electron microscopic study. *J. Biophys. Biochem. Cytol.*, (in press).
9. Hawn, C. V. F. and Porter, K. R.: The fine structure of clots formed from purified bovine fibrinogen and thrombin: A study with the electron microscope. *J. Exp. Med.*, **86:** 285, 1947.
10. Hall, C. E.: Electron microscopy of fibrinogen and fibrin. *J. Biol. Chem.*, **179:** 857, 1949.
11. Low, F. N. and Freeman, J. A.: Electron microscopic atlas of normal and leukemic human blood. New York, McGraw Hill, 1958.
12. Waksman, B. H.: A comparative histopathological study of delayed hypersensitivity reactions. Pages 280-322. In, Ciba Foundation Symposium on Cellular Aspects of Immunity (Eds. G. E. W. Woltstenholme and M. O'Connor) London, Churchill, 1960.
13. Wilgram, G. D., Caulfield, J. B. and Lever, W. F.: An electron microscopic study of acantholysis in pemphigus vulgaris. *J. Invest. Derm.*, **36:** 373, 1961.